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THE FUNCTION OF TANNIN IN HOST-PARASITE RELATIONSHIPS WITH SPECIAL REFERENCE TO RIBES AND CRONARTIUM RIBICOLA

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CONTENTS

INTRODUCTION

The purpose of this circular is to summarize unpublished data 1/ on the tannin content of Ribes spp., obtained in the course of laboratory and greenhouse investigations on the chemical eradication of ribes (36, 37). 2/, 3/

The original objective of the laboratory and greenhouse work, undertaken during the period 1927-1930, was to determine whether plant extractives such as tannins served as protective agents against the toxic action of herbicides. Evidence was obtained to show that, in general, the most effective herbicides were those which form soluble compounds or mixtures with tannins and similar water extractives rather than those which form immediate precipitates with such extractives.

As a by-product of this work, it was noted that the leaves of the black currants, which are highly susceptible to blister rust disease (Cronartium ribicola Fischer), tended to be low in tannin, while some of the resistant species, such as Ribes lacustre, showed a high tannin content. Laboratory tests on the nature of the tannins in the leaves of R. petiolare and R. inerme--two ribes differing in susceptibility to the rust--indicated that there were differences in the type as well as the quantity of tannin. It was realized that both quantity and quality of the tannins could be significant factors in resistance to a fungous disease.

1/ The tannin analyses were made at Berkeley, Calif., chiefly by G. R. Van Atta, and at Moscow, Idaho, by R. P. d'Urbal. Research facilities were made available at Berkeley through the cooperation of the Department of Forestry and the Division of Plant Nutrition of the College of Agriculture, University of California, and at Moscow through the Forestry Department of the University of Idaho. Microchemical tests were made at Berkeley by I. E. Webber, with the assistance of F. A. Patty and C. R. Quick, and were made possible through facilities furnished by the Department of Botany, University of California. At the time that the work on tannin was undertaken, the blister-rust-control activities were directed by the Bureau of Plant Industry. Since December 1933 this work has been directed by the Bureau of Entomology and Plant Quarantine.

2/ The generic name Ribes and the common name ribes are used in this paper to indicate both currants and gooseberries.

3/ Numbers in parentheses refer to the literature cited at the end of this circular.

Since the writer was primarily concerned with development of herbicides and not with research on the physiological relations between ribes and the blister-rust fungus, the investigations were dropped when they were no longer of immediate use to practical work on the chemical eradication of ribes. Recently, however, there have been a number of requests for a summary of the data on the tannin content of ribes obtained during the course of this work. These requests reflect the increasing interest in the physiological function of tannins and their decomposition products, especially in pathologic relations between host and parasite (6, 10, 12, 14, 29, 55), in horticultural and ecological problems dealing with crop rotation and plant succession (9, 15, 46), and in matters pertaining to the palatability and nutritive value of forage (4, 7).

Recently published data (14, 25, 29, 34, 35) show that the degradation products of tannins are the substances most directly concerned in the protective action against fungi. Since these products have not been thoroughly studied and identified, it seemed preferable to make general use of the term tannin throughout this report.

In order to indicate the significance of the data on the tannin content of Ribes spp., the chemical and physiological properties of tannins and related work on the role of tannins in host-parasite relationships will be presented first.

PREVIOUS RELATED INVESTIGATIONS

Chemical properties of tannins

It has been customary to apply the name tannin to that portion of the water-soluble matter of certain vegetable materials which will precipitate gelatin from solution and which will form compounds with hide fiber that are resistant to washing. In this paper, tannin is used as a collective term for a group of substances having the special properties just described.

Tannins are amorphous or crystalline solids of astringent taste, having wide occurrence and general distribution in the tissue of most higher plants. They are readily obtained from pulverized or shredded plant material by extraction with hot water or alcohol. Tannins exhibit a marked tendency to absorb oxygen, especially in alkaline solution. Fischer (17) synthesized penta-m-digalloyl-beta-glucose, and showed it to be an isomer of the tannin obtained from Chinese nutgalls, and some recent work by Russel and coworkers (47, 48, 49, 50) has extended data on the chemical structure of tannins. The greater part of the work on tannins, however, has dealt chiefly with methods for classifying, extracting, and measuring tannins for use in the leather industry. Classifications generally accepted are those of Perkin (38) and Freudenberg (18).

The arrangement of tannins into classes is based on color reactions with ferric salts and other reagents, on the presence or absence of a precipitate with bromine water, on the production of a so-called "bloom" on leather, and on the products of hydrolysis. For the discussion presented in this paper, Perkin's 3-group classification will be used, i.e., alpha group (depsides or gallotannins); beta group (ellagitannins); and gamma group (phlobatannins or catecholtannins). The following decomposition products of tannins are of major importance in their classification:

(1) Heated alone, they form trihydric phenols (pyrogallol) and dihydric phenols (catechol).

(2) Heated with dilute acids, they form dextrose, trihydroxyacids (gallic acid), ellagic acid, and insoluble amorphous anhydrides called phlobaphenes.

(3) Fused with alkaline hydroxides, they form trihydric phenols (pyrogallol and phloroglucinol), dihydroxyacids (protocatechuic acid), and acetic acid.

The function of tannins in plants

Although physiological investigations of the role of tannins in plant life have been hampered by incomplete data on their chemical nature, it has long been recognized that tannins are something more than by-products in plant metabolism, and a physiological function has been assigned to them by most workers (10, 14, 25, 31, 39).

Tannins have been noted to affect the activity of enzymes. Brown and Morris (5) observed that tannin inhibited the action of diastase in leaves, and Vinson (56) showed that the solubility of date invertase increased as the fruit ripened, and that these changes were paralleled by changes in the solubility of the extractable tannins. Tannin has also been used to absorb peroxidase (16).

Moore, as noted by Haas and Hill (19), states that tannins may play an important part in the lignification of cell walls and are tied up with cork formation, and further that tannin in the epidermis of leaves may influence the opening and closing of stomata. Denny (13) treated seeds of pumpkin, almond, and peanut with solvents for tannins and lipoids, and reported that tannin is one of the ingredients of the cell wall which affects its permeability to water. Lloyd (29) and Herszlik (24) present evidence to show that in the cell sap and vacuoles tannin is linked up or adsorbed with cell constituents of a cellulose nature and that tannin vacuoles in cortical tissue are protected by a pectic membrane from contact with protoplasm.

Quendiac (41), in his studies on oak trees, concluded that early in spring tannins are localized in areas in which sap flow is restricted, notably the parenchyma of the sapwood. In May the accumulation zone extends toward the ends of the branches. Subsequently the sapwood develops two distinct regions, one normal and one with tannins, the accumulation corresponding with the zone of transition between heartwood and sapwood.

The most comprehensive study of the location and function of tannins in the plant cell is that of Dekker (12), who used ribes as experimental material. Using a 5-percent potassium bichromate solution as a stain, he studied the distribution of tannin in leaves, current-season twigs, old stems, fruits, and roots. He concluded that tannin accumulated in regions of strong vegetative growth--for example, in the tips of twigs. Tannin invariably occurred in those layers of cells which, because of their position, may be expected to have a protective function. In the young stalk this is the epidermis and its bordering layers; in the older stem and roots it is the cork tissue. In Ribes sp. the primary vascular bundles in the earliest stages of the stalk are surrounded by a girdle of cells which are particularly rich in tannin. By covering active ribes leaves with tinfoil and gelatin paper, and comparing the tannin-bichromate color in covered leaves with uncovered controls, Dekker showed that light was an indispensable factor in the formation of tannins in the leaf. The results of further staining tests showed that the tannin bands in the medulla are of significance in the transport of reducing sugars.

According to Hauser (21, 22, 23), tannins act on the cell plasma as polyvalent phenols, with relatively large molecules tending to prevent the formation of coarse dispersions and secondary structures. He ascribes a regulatory function to tannin corresponding to that of a protective colloid. The effectiveness of this protective colloid will depend upon its degree of dispersion and upon related phenomena, such as permeability, absorptive power, and cell turgor.

Influence of tannin on host-parasite relations

The high concentration of tannin in pathologic galls and in hypertrophied tissue resulting from insect injuries called early attention to the role of tannins as probable protective materials. Ward (57), Cook and Taubenhaus (10), and others listed tannins among the probable substances having a chemotactic action on the germ tubes of fungi. Cook and Taubenhaus conducted extensive tests on the toxicity of tannin on a wide variety of organisms. They point out the necessity of correlating the function of tannins with other constituents of the plant cell in any consideration of the reaction of a plant to invading fungi.

Cook and Taubenhaus also showed that small amounts of tannin may stimulate production of spores and that specificity of action between tannin and the various invading fungi was indicated. They show that Fusariums were more resistant that Gloeosporiums and Collectotrichums; the Cladosporiums were more resistant than the Fusariums, and Penicillium oliveceum was the most resistant of all species tested. The majority of parasites were retarded by 0.1 to 0.6 percent of tannin.

The fact that plants which contain unusually high percentages of tannin may also be subject to disease indicates that tannin per se cannot be exclusively charged with the protection of a plant against fungi. The chemical nature of the tannins, the quantity present, the location of the tannin-bearing cells, the available food supplies near the point invaded by the parasite, and the effect of enzymes or hormones on the liberation of protective substances must all be considered.

In a report of cytological studies on the biochemical aspects of immunity, Dufrénoy (14) points out that the susceptibility of a plant to invading hyphae depends upon the death rate of the attacked cells, and the ability of the germ tubes to establish new contacts with adjoining cells. He states that in a resistant plant the attempt at penetration by a filament rapidly provokes the formation of phenolic compounds,4/ which results in death of the first cells threatened by the infection. Thus the filament is unable to establish a relation with the plant that it is trying to invade. In highly susceptible plants the filaments probe between the living cells and penetrate them without causing a significant break-down in the cell plasma. If the fungus grows rapidly enough to establish itself beyond the zone of cells containing toxic concentrations of phenolic compounds, it can continue its advance. Conversely, development of the fungus is arrested if it cannot grow fast enough to keep established beyond the zone of differentiated cells.

Rose (44, 45) reported that apple bark carrying blister canker caused considerably more oxidation with pyrocatechin, guaiacol, and benzidine than healthy bark, and attributed the phenomenon to the influence of tannin on oxidase activity.

4/ In a report before the American Association for the Advancement of Science, G. A. Greathouse and N. E. Rigler "showed that natural and derived phenolic compounds are toxic to the root-rot fungus. Isomerism was found to play an important part in toxicity." Science, vol. 91, p. 116, Feb. 2, 1940. Published later in Amer. Jour. Bot. 27: 99-108 (1940).

The specificity of phenolic combinations (tannins) in reactions with invading fungi, the high toxicity of certain types of these phenolic combinations, and their propensities for oxidation-reduction action in the plant cell have been confirmed and further developed by the work of Kargapolova(25). He investigated wheat species and varieties extremely resistant or susceptible to Puccinia triticina for their phenolic content and derivative substances. Susceptible varieties contained mere traces, or only about one-third to one-quarter as much of the phenolic compounds as the resistant varieties. Qualitative reactions showed that the tannin fraction contributing most strongly to immunity was of the pyrocatechin rather than the pyrogallol type. It is known that the pyrogallol series of tannins are readily hydrolized by enzymes (such as those carried by yeasts and fungi) into nontoxic materials, whereas the pyrocatechin series are more stable, and are thus more persistent in their protective and precipitating reactions on proteins. Kargapolova's tests of the effective action of pure phenols on the spores of P. triticina also showed that the separate phenols vary in toxicity. Of the phenols tested, the most toxic for the rust were hydrochinon and pyrocatechin. The acetic-ethyl fractions, when tested directly on spores of P. triticina, were more toxic when prepared from immune than from susceptible varieties of wheat plants.

Thornberry (55) reported that tannic acid, if applied prior to inoculation, inhibited the tobacco mosaic in direct proportion to its concentration. Stapp and Bortels (54) noted that not all fungi were able to split tannins; in their tests the ability to decompose tannins was restricted to several types of Aspergillus and Penicillium. Rippel and Keseling (43) report that Penicillium, Citromyces, and Aspergillus spp. were the only molds examined that were able to use tannin as a sole source of carbon. Bavendamm (4) and Davidson(11) describe methods for the differentiation of wood-decaying fungi, based on their growth reactions on media containing gallic or tannic acid.

Naumann (32) described experiments to show that: "Fungi cannot produce tannin, but may take it up and utilize it as food when decomposed. Certain fungi do not absorb tannin and are injured in presence of excessive amounts. Polyporeae contained 0.034 to 0.400 percent tannin and Agaricaceae 0.041 to 0.060 percent tannin. Parasites usually contain more tannin (0.180 to 0.40). Tannin is chemically decomposed in fungi."

Specific physiological responses following fungous attack are also indicated by transpiration phenomena. Reed and Cooley (42) reported that apple leaves infected with Gymnosporangium juniperi-virginianae showed lower transpiration loss than healthy leaves. Lowered transpiration for cocklebur infected with Puccinia xanthii was reported by Weaver (58). There appear to be many instances, however, where fungous attack is followed by a marked increase in transpiration rate. Weaver (58) has suggested that increased transpiration on the part of infected plants might be attributed to changes in the permeability of cell walls brought about by substances secreted by the fungus.

The physiological nature of host-parasite relations in blister-rust disease

The resistance of the host plant to an attacking parasitic fungus is generally physiological rather than anatomical in nature. Early work by Ward (57) and more recent work by Dufrénoy (14), Kargapolova (25), Stakman (53), and Allen (1) have established this observation for a wide variety of plants and fungi.

The same conclusions were reached by the writer, following a histologic study 5/ of the internal and external anatomy of several ribes of widely differing susceptibility, such as the highly susceptible R. petiolare and the immune Viking red currant (syn. Rød Hollandsk Druerips.). The number of stomata, the thickness of protective tissue, and other histologic characters frequently varied as much between ecologic forms of one species as between resistant and susceptible varieties.

The recent work of Anderson (2) provides confirmatory evidence that the resistance of Ribes sp. to Cronartium ribicola is physiological in nature.

In a cytological study of the immune Viking currant Anderson found (2) that the entrance phenomenon of the hyphae was essentially the same for the immune currant and for the susceptible Ribes nigrum, R. sativum, and R. hirtellum, Stakman (53), Newton (33), and Allen (1) also report no essential difference in entrance phenomena of the rust hyphae in resistant and susceptible varieties of wheat attacked by Puccinia graminis Pers. and P. graminis tritici Erikss. Anderson (2) confirmed the previous conclusions of Hahn (20) that the blister-rust fungus was able to enter the leaves of a Viking currant but failed to propagate itself. About 48 to 72 hours after inoculation of the Viking currant, the contents of the invading hyphae showed vacuoles and became granular, providing evidence of marked degeneration. Within 96 hours the mycelium had broken down and disappeared.

Investigators working with resistant wheat plants (1, 33, 53) report that the death of host cells precedes that of the parasitic hyphae, with the resultant death from starvation of the invading organisms. In the Viking currant, Anderson observed that the death of hyphae preceded that of the host cells by several days, and for this reason he postulates a physiological incompatibility.

5/ See unpublished reports on the morphology of Ribes sp. by I. E. Webber, C. R. Quick, and F. A. Patty on file with the Bureau of Entomology and Plant Quarantine, Division of Plant Disease Control at Berkeley, Calif. and Washington, D. C.

The differences noted by Anderson in the response of young, fully expanded leaves and fully matured, hardened leaves also point to the physiological nature of resistance. Germ tubes penetrated the leaves through stomata in both types of leaves, but only the young leaves permitted the temporary development of hyphae. The chemical and physiological properties of tannins, as previously pointed out, are such that quantitative and qualitative differences may be assumed in various plant parts where differences occur in age and metabolic activity. That the susceptibility of ribes leaves and pine needles to blister rust varies with the age of leaves and needles has previously been demonstrated by the work of Spaulding (52), Snell (51), Lachmund (27), and Mielke (30). For ribes, Spaulding (52), summarized many of these data as follows:

> "The age and relative maturity of the leaf has much to do with its susceptibility. It has been the general experience that Ribes leaves may be overmature and also may be too young to take the disease. Infection does not occur on leaves of a given species of Ribes until they have reached a certain degree of maturity. Leaves produced by buds developing in late summer or fall even if very small, readily become infected. The different species of Ribes vary much in this regard. Ribes nigrum shows a great range in its age of susceptibility, while resistant species become infected only on leaves of a certain maturity. The most favorable stage of growth seems to be about when the leaf attains full size but has not become hardened and leathery as it does later."

Under field conditions it is difficult to establish with any degree of accuracy an order of susceptibility of ribes to blister rust disease, especially if the list is intended to cover ribes of different geographic and ecologic regions. Kimmey (25) showed that susceptibility to direct inoculation varied among species and even in the same species for open, part-shade and shade forms. The ability to produce telia presented a different order of susceptibility than that provided by the concept of ability to take infection, and the ecologic influences were not always followed by a consistent increase or decrease in reaction to the disease. Generally the more susceptible plants produced the most telia, and vice versa. Within a species, the part-shade form was the most susceptible and produced the most telia; the open form was least susceptible and produced the least telia. This general reaction to the rust is consistent with the physiology of tannin production in the plant, inasmuch as sunlight and high plant metabolism tend to optimum production of tannin.

Hahn (20) has recently made an interesting contribution to studies on the susceptibility of ribes to blister rust disease, by demonstrating the immunity of a staminate clone of Ribes alpinum. This observation would indicate that susceptibility is sex-linked, and that a hormone or enzyme may be responsible for initiating subsequent protective responses of the plant.

Status of the tannin question as it relates to disease resistance

In presenting the foregoing discussion, the purpose has been to assemble data from published records which relate to the chemical and physiological properties of tannins and to the effect of tannins and their degradation products on the growth of fungi. No attempt has been made to discuss generally the biological aspects of disease resistance in plants. A recent review of this subject by Brown (6) shows all too clearly the size of such a task and the mass of data--most of it analogous in nature--which might be taken as support for one of several theories.

The case for the tannins must certainly be considered in the light of their widespread distribution and occurrence, frequently in large amounts, in plants which are readily attacked by fungi. On the other hand, the toxicity of many tannin extracts, as expressed in their precipitating action on albuminoids and in direct tests on the growth of parasitic fungi, has been well enough established to be generally accepted. The presence of tannin in solution in the vacuoles of the normal plant cell, however, would presumably require (a) that the protoplast be protected against immediate contact with the reactive tannin by a limiting layer of a secondary adsorbed compound, or (b) that the concentration of tannin in solution be too low to precipitate albuminoids or (c) that the toxicity of tannin be indirect and caused by degradation products released through enzymic action upon contact of host and parasite.

In the writer's opinion, the mechanism of the tannin-parasite relationship, in agreement with most of the direct evidence, is the following: An enzyme or hormone secreted by the fungus initiates the penetrative action into living cells. The first barrier to successful contact of haustoria and cell contents is the adsorbed tannin of the cell wall. At this point the complex nature of the tannins offers plenty of scope for specificity based on the lock-and-key simile of enzyme and substrate.

If the chemical composition of the pectic and tanninlike substances of the cell wall cannot be converted into soluble or usable decomposition products by the enzyme or hormone secreted by the fungus then the haustoria fail to develop and these specialized feeding branches die while the plant cells are still turgid. This type of resistance is probably that noted by Anderson (2) in the case of the immune Viking currant. Should the haustoria be able to penetrate into the plant cell, then the soluble and oxidizable tannins of the cell sap may offer usable nutrients to the fungus, with the resultant stimulation of its growth (10, 11, 32, 43). Or, the tannins may form ingredients upon decomposition which are toxic to fungi (10, 14, 25). Failure of the haustoria to develop, or disorganization and death of the plant cells prior to that of the haustoria as noted by Stakman (53) and Allen (1), would seem to depend on whether the antagonistic action is exerted at the surface layer of the plant cell or within the cytoplast. Following contact of the haustoria with cell contents, subsequent growth and development of the invading organism may be retarded or encouraged by the nature of the toxic or oxidizable tannins occurring in solution in the sap vacuoles.

The salient facts just summarized may now be used to interpret the results of the tannin studies made by the writer.

SUMMARY OF DATA ON TANNIN CONTENT OF SEVERAL WESTERN RIBES

Qualitative tests on the tannins of Ribes petiolare and R. inerme

Laboratory investigations conducted at Berkeley in the fall of 1927 showed differences in the chemical properties of constituents of the tannin aggregate in R. petiolare and R. inerme. The tannins of R. petiolare belong to the group that gives blue-black precipitates with ferric salts, while R. inerme tannins give a mixture of both blue-black and green precipitates. On careful heating, R. petiolare tannin showed the characteristic flakelike crystals of pyrogallol, while R. inerme tannin changed over into a black amorphous mass characteristic of the phlobaphenes. Acid hydrolysis of R. petiolare tannin yielded only a trace of dextrose, considerable gallic acid, and some phlobaphene. Acid hydrolysis of R. inerme tannin showed a fair quantity of dextrose, no gallic acid, and a large amount of phlobaphene. Fusion with potassium hydroxide indicated pyrogallol in the case of the former and phloroglucinol for the latter. During the course of a proximate analysis of the leaves and stems of R. petiolare, R. inerme, R. viscosissimum, and R. lacustre, it was noted that less than 1 percent of the total tannin of R. petiolare was obtained in an alcohol fraction, while about 50 percent of the tannin in R. lacustre, R. inerme, and R. viscosissimum was removed by alcohol. Thus it is seen that the tannin complex of ribes comprises ingredients which differ widely in chemical composition and physical character.

On the basis of these tests it appears that Ribes petiolare tannins belong chiefly to the alpha group containing the depsides or gallotannins (Perkin's classification) which yield phenolics of the pyrogallol type, while the R. inerme tannin complex contains representatives of both the alpha and gamma types. As Kargapolova pointed out, the pyrogallol phenols and their tannins (alpha group) are readily hydrolyzed by many enzymes of fungi into alcohols and nonactive phenol acids and are therefore less reliable protective substances than the catechol tannins (gamma group), which are less easily hydrolyzed by enzymes and which thus rate in their power of precipitating proteins.

Histological work

The histologic methods used by Dekker (12) were employed at Berkeley in the winter of 1928 to study the distribution of tannin in Ribes petiolare Dougl., R. lacustre (Pers.) Poir, R. viscosissimum Pursh., and R. inerme Rydb. Leaves and young stems of greenhouse-grown plants were sectioned, stained with 5-percent potassium bichromate, and photomicrographic records were made. In noting the distribution of tannin and allied substances, it was observed that--

(a) In leaves such substances may occur in epidermal cells, epidermal hairs, scattered cells of palisade and spongy parenchyma, border parenchyma, and vascular tissues.

(b) In young stems such substances may occur in the epidermis and its appendages, scattered cells of the cortex, the pericycle, phloem, scattered cells of the xylem, and scattered cells of the pith.

(c) The following data pertain to leaves: Ribes petiolare showed traces of tannin in the epidermal layers and only small amounts in the vascular tissue of the midrib; R. lacustre and R. viscosissimum had tannin rather evenly distributed in epidermal layers and spongy parenchyma; R. inerme showed less tannin in epidermal layers than R. lacustre and R. viscosissimum and about equal amounts in the spongy parenchyma.

The amount of such substances present was not constant for any of the species. Free-hand sections of leaves and stems taken from greenhouse ribes and stained for tannins with potassium dichromate showed considerable variation in amount of tannin between individuals as well as between species. Variation was apparently due to several factors, of which differences in illumination seemed to be one.

Dekker's experiments with tinfoil-and gelatin-covered leaves were repeated, using several sets of leaves from different positions on a number of plants. After 48 hours under artificial illumination, the leaves were stained in bichromate solution, and in every case the gelatin-covered and the uncovered leaves were darker (showing the presence of more tannins) than the tinfoil-covered leaves. Fresh ribes leaves taken from the plants following several cloudy days were much lower in tannin content than the field samples first tested. After two sunny days, R. lacustre showed an increase in the amount of tannin as indicated by the dichromate staining reaction.

An attempt was made to study the quantitative distribution of different types of tannin by immersing freshly picked ribes leaves in various solvents, prior to staining with potassium bichromate. Observations from these tests are summarized below.

Microchemical tests for tannins on greenhouse ribes

Leaves gathered at end of day of full sunshine from mature plants grown on sand cultures:

R. petiolare--

(1) Directly into the bichromate.
Tannin reaction in the vascular tissue rather marked, quite well marked through the spongy parenchyma, and noticeable in some cells of both upper and lower epidermis. Practically none in the palisade parenchyma.

(2) Directly into absolute alcohol for 24 hours, then into bichromate.
Tannin reaction slight in the phloem region. Not noticeable elsewhere.

(3) Directly into water, kept at 54° F. for 24 hours, then into bichromate.
Tannin reaction as in (1) above, but less marked, particularly in the spongy parenchyma.

R. lacustre--

(1) Directly into bichromate.
Tannin reaction exceedingly well marked in the region of the vascular tissue and in a few cells throughout spongy parenchyma and palisade cells. Parenchyma cells give fairly well marked reaction in lower and upper epidermis.

(2) Absolute alcohol and then bichromate as above.
Tannin reaction well marked in vascular tissue; a few cells in spongy parenchyma and some palisade cells give fairly well marked reaction.

(3) Water and bichromate as above.
Slight tannin reaction in the vascular tissue, practically none elsewhere.

R. inerme--

(1) Directly into bichromate.
Fairly well marked tannin reaction in the vascular tissue, practically none elsewhere.

(2) Absolute alcohol and bichromate as above.
Slight tannin reaction in the vascular tissue.

(3) Water and bichromate as above.
Slight tannin reaction in vascular tissue.

Leaves gathered late in the afternoon from young, vigorous plants exposed to sunlight plus artificial illumination. Plants grown on water culture:

R. inermc--

(1) Directly into the bichromate.
Tannin indicated by strong staining reaction in upper and lower epidermal cells, glandular hairs, border parenchyma, a few scattered cells in phloem, radial rows of cells in the xylem, scattered cells in palisade and spongy parenchyma.

(2) Directly into water, kept for 24 hours, then into bichromate.
Tannin indicated by medium orange color in glandular hairs, pale orange in a few epidermal cells and occasional palisade cells.

(3) Directly into ethyl acetate for 24 hours, then into bichromate.
Tannin indicated by medium orange-brown coloration in scattered phloem cells, radial rows of cells in xylem, and rather pale orange-brown coloration into border parenchyma.

R. petiolare--

(1) Directly into bichromate.
Tannin indicated by deep orange-brown coloration of upper and lower epidermal cells, in scattered subepidermal and parenchyma cells in region of the midrib, border parenchyma, scattered cells in phloem and radial rows of cells in xylem, few scattered cells in palisade parenchyma and numerous cells in spongy parenchyma.

(2) Water and bichromate as above.
Light orange-brown color indicates tannin in scattered phloem cells and radial cells in xylem.

(3) Ethyl acetate and bichromate as above.
Tannin indicated by orange coloration in glandular hairs. Orange-brown scattered cells in spongy and palisade parenchyma, rather deep orange-brown coloration in border parenchyma, in phloem cells, and radial rows of cells in xylem.

R. lacustre--

(1) Directly into the bichromate.
Tannin indicated by orange-brown coloration in epidermal cells, glandular hairs, border parenchyma, phloem cells, and radial rows of cells in the xylem.

(2) Ethyl acetate and bichromate as above.
Tannin indicated by brownish coloration in the vascular tissue.

These tests showed that young, fully developed leaves contained more tannin than older, hardened leaves, and further confirmed the importance of light for optimum production of tannin. The intensity and actual distribution of the stains indicated that the solvents water, absolute alcohol, and ethyl acetate had reacted differently. These tests merely confirm the differences previously noted in the chemical properties of the tannin aggregate previously noted for R. petiolare and R. inermc.

Quantitative determinations of tannin for several western ribes

In September 1927, leaves of Ribes petiolare, R. inerme, R. viscosissimum, and R. lacustre were collected from several areas within the white-pine belt of northern Idaho. These leaves were air-dried on wire trays and subsequently analyzed for total tannin. The following year fresh leaf and stem material of the same four ribes species was collected and preserved in alcohol for proximate analysis.

In 1930 a more comprehensive collection of ribes material was made in northern Idaho, western Oregon, and central California. Leaves, current-season stems, old stems, and roots were separately collected, air-dried in the shade on wire screens, and then packed in paper bags for subsequent tannin analysis.

The official hide powder method (3, pp. 119-129) was used for all of the tannin analyses reported in tables 1 and 2. Fixable tannin was determined by the revised Wilson-Kern method (59, pp. 290-295) for several samples from the 1928 and 1930 collections, to compare the quantities of so-called fixable and total tannin. The total tannin (official method) was from 30 to 50 percent higher than the fixable tannin (Wilson-Kern method) for all the samples tested. Since the latter data are incomplete they will not be included in this report.

For the collections made in 1930, an attempt was made to obtain a representative sample containing about equal amounts of healthy plants of both shade and sun forms. While it was realized, from preliminary tests made in 1928 and 1929, that there would be considerable variation within a single species for shade and sun forms of that plant, the purpose of the 1930 survey was to obtain quantitative data which could be taken as indicative of the species as a whole.

For the collections made in 1930, detailed reports on file at Berkeley show weather data, and remarks on insolation, age, and vigor of bushes, as well as notes on site and associated vegetation. These field notes add nothing to the possible interpretations from the quantitative data shown in table 1 and are therefore omitted from this report.

The results of the tannin determinations for the 1930 collections and a summary of data for the tannin content of the leaves of the 1927, 1928 and 1930 collections of the four principal ribes of northern Idaho are given in tables 1 and 2. In all cases the figures shown are averages for at least two determinations.

Table 1.--Seasonal variation in tannin content of leaves, stems, and roots of several Ribes species

Species	Date of collection	Portion of plant analyzed[1]/	Tannin content[2]/ (bone-dry basis)
			Percent
Ribes petiolare[3]/	1930 June 2,3,4	L	5.87
		CSS	3.93
		OS	3.07
		R	4.07
Do.	July 25	L	5.94
		CSS	3.74
		OS	2.19
		R	4.00
Do.	Aug. 29	L	4.06
		CSS	1.29
		OS	0.92
		R	3.64
R. inerme[3]/	1930 June 3, 4	L	5.52
		CSS	0.43
		OS	2.14
		R	3.55
Do.	June 23,24, 25,26	L	5.85
		CSS	0.65
		OS	2.26
		R	1.53
Do.	July 21	L	3.12
		CSS	0.75
		OS	0.61
		R	1.33
Do.	Sept. 1	L	3.49
		CSS	0.89
		OS	1.04
		R	2.92

1/L = leaves, CSS = current-season stem, OS = old stem, and R = roots.

2/Each figure shown is the average of two determinations.

3/Collected at Renfro Creek, Santa, Idaho.

Table 1 (contd.)

Species	Date of collection	Portion of plant analyzed[1]/	Tannin content[2]/ (bone-dry basis)
			Percent
R. viscosissimum[3]/	1930 July 2	L	11.30
		CSS	8.23
		OS	2.12
		R	7.00
Do.	July 25	L	9.75
		CSS	5.70
		OS	3.12
		R	5.61
Do.	Sept. 1	L	8.79
		CSS	3.51
		OS	2.01
		R	4.16
R. lacustre[3]/	1930 June 26,27, 28	L	16.70
		CSS	5.74
		OS	1.61
		R	4.85
Do.	July 23	L	17.95
		CSS	3.74
		OS	1.54
		R	4.28
Do.	Sept. 1	L	18.00
		CSS	1.83
		OS	1.21
		R	4.04
R. nevadense[4]/	1930 June 10	L	12.10
		CSS	5.22
		OS	4.67
		R	9.20
Do.	July 22,23	L	12.72
		CSS	8.78
		OS	3.96
		R	7.66
Do.	Sept. 9	L	11.29
		CSS	6.93
		OS	5.22
		R	4.43

4/ Collected at South Fork of Stanislaus River below Strawberry, Stanislaus N. F., Calif.

Table 1 (contd.)

Species	Date of collection	Portion of plant analyzed[1]/	Tannin content[2]/ (bone-dry basis)
			Percent
R. roezli[5]/	1930 June 11	L	12.71
		CSS	4.04
		OS	2.47
		R	4.48
Do.	July 16, 17	L	12.80
		CSS	6.85
		OS	4.47
		R	9.22
Do.	Sept. 1	L	13.58
		CSS	6.15
		OS	4.32
		R	7.71
R. bracteosum[6]/	1930 Aug. 18	L	5.11
		CSS	2.64
		OS	2.29
		R	8.27
R. erythrocarpum[7]/	1930 Aug. 20	L	16.95
		CSS	--8/
		OS	4.76
		R	7.50
R. lacustre[6]/	1930 July 22	L	14.93
		CSS	4.13
		OS	0.72
		R	4.58
Do.	Sept. 6	L	15.21
		CSS	1.27
		OS	1.02
		R	1.92
R. nigrum[9]/	1928 June 12	L	4.70
R. alpinum[10]/	1928 May 28	L	8.80

5/ Collected at Cow Creek, Stanislaus N. F., Calif.
6/ Collected at Still Creek, near Swim, Oregon.
7/ Collected at Annie Creek, Rogue River N. F., Oreg.
8/ The amount of current-season stem was so small that it could not be readily collected.

Table 2.--Tannin content of the leaves of four principal Ribes species of northern Idaho

Species	Date of collection	Number of collections	Method of preserving field specimens	Tannin in leaves[1/] (bone-dry basis)
				Percent
R. petiolare	Sept. 1927	1	Air dried	1.6
Do.	May 1928	1	In alcohol	4.9
Do.	June 4, July 25, Aug. 29, 1930	3	Air dried	5.29
R. inerme	Sept. 1927	1	Air dried	3.4
Do.	May 1928	1	In alcohol	7.9
Do.	June 3,23; July 21; Sept. 1, 1930	4	Air dried	4.50
R. viscosissimum	Sept. 1927	1	Air dried	11.9
Do.	May 1928	1	In alcohol	9.2
Do.	July 2,25; Sept. 1, 1930	3	Air dried	9.95
R. lacustre	Sept. 1927	1	Air dried	13.8
Do.	May 1928	1	In alcohol	11.2
Do.	June 26, July 23, Sept. 1, 1930	3	Air dried	17.55

1/ Tannin figures shown for the 1927 and 1928 collections are averages of duplicate tests; data for the 1930 materials are seasonal averages taken from table 1.

Data in table 1 show that, with a few exceptions, the tannin content of ribes leaf tissue, current-season stem, root, and old stem decreases for the various plant parts in the order named. Over the period of collection (June to September) no substantial variation is shown in the tannin content of leaves. The last collections for the Idaho and California Ribes species were made just prior to the beginning of leaf fall when leaves were mature and well hardened. In no case were leaves collected early enough to provide a correlation with the observations of Lachmund (28) regarding the high susceptibility of young leaves to blister-rust disease. Generally speaking, there is a slight reduction in the tannin content of ribes leaves after midseason.

Insofar as host-parasite relationships for ribes are concerned, the tannin content of the leaf would be most directly involved in any physiological reactions. Attention is therefore directed to leaf-tannin data. If all the ribes listed in table 1 are taken as a group, there is no direct correlation between the quantitative tannin data and known susceptibility to blister-rust disease. Although the highly susceptible black currants Ribes petiolare, R. bracteosum, and R. nigrum are generally low in tannin and the highly resistant R. lacustre (Oregon and Idaho) is the highest in tannin, some of the other species are out of line. Most noticeable is R. roezli (one of the most susceptible of the ribes listed in table 1), which contained 12 percent of tannin, R. alpinum is generally conceded to be a resistant ribes on the order of R. lacustre, and in table 1 it occupies an intermediate position in regard to tannin content. There is, however, a recent report by Hahn (20) in which he shows that the susceptibility of the dioecious R. alpinum varies significantly between the staminate and pistillate forms. The leaf sample of R. alpinum sent to the writer presumably contained material from both staminate and pistillate plants. It would be interesting to compare the quantity and nature of the tannins in leaves taken from staminate and pistillate specimens of R. alpinum.

Data for the four principal species of northern Idaho, as summarized in table 2, are more complete than those for the remaining species, and comparative susceptibility to blister-rust disease has been most clearly established for these species. Mielke and coworkers (30) show that Ribes petiolare and R. inerme are much more susceptible than R. viscosissimum and R. lacustre. Under natural conditions, R. viscosissimum has been more active in spreading and intensifying the rust in northern Idaho than R. inerme, but factors other than direct susceptibility have combined to bring this about. For these four species, quantitative data for leaf tannin show a general correlation with susceptibility to blister-rust disease. For the complete group of ribes studied, however, the quantity of tannin in leaf tissue cannot be taken as a direct measurement of susceptibility to blister-rust disease.

Variations in the quantity and chemical properties of tannins in the Ribes species described in this report indicate that the tannins would be a profitable subject for further investigative work on the susceptibility of ribes to Cronartium ribicola. Data from such a study would bear on the highly important matter of the mechanism of disease resistance in plants. In this field of work Ribes species and Cronartium ribicola would be convenient experimental material for the following reasons: A pure stock of experimental plants can be maintained, because ribes are readily propagated from cuttings and may be conveniently cared for under greenhouse conditions. Detached ribes leaves may be preserved on nutrient solutions in petri dishes for use in inoculation tests according to the technique described by Clinton and McCormick (8). For reaction tests the complete range of susceptibility to the rust is provided by the highly susceptible black currant on the one hand and the immune Viking currant on the other. The racial purity of the aeciospores used to infect ribes could be maintained. Finally, a vast amount of data is already available on the susceptibility of ribes and pine under field conditions, and on the histology and ecology of ribes.

Although blister rust is generally conceded to be heterothallic (40), the question of whether biotypes in the blister-rust fungus do exist has never been answered satisfactorily. This point has not been of great importance in large-scale blister rust control work because so far it has not been practical to propagate and plant resistant varieties of white pine. Nevertheless a careful technical study of the susceptibility of ribes could not be undertaken without the assurance that leaves were being infected by a pure strain of aeciospores. Because of the difficulties that have been experienced experimentally in preventing the urediospores from going into telia, it would probably be necessary to maintain the purity of the rust strains on ribes by making single spore inoculations. Such a study also would be advisedly carried out in regions where the disease is already established, and where there would be no hazard from the control standpoint in having the work in progress.

SUMMARY

Evidence shows that the chemical properties of tannins and their degradation products, their probable function in the plant, and their action on parasitic fungi lend themselves to an explanation of the specificity exhibited in host-parasite relationships. A theory is offered which postulates a toxic action of tannin initiated and conditioned by enzymes or hormones secreted by the fungus. The ultimate toxicity of the tannins would appear to depend partly on the type of phenolics and other potentially toxic constituents formed by the reaction of host and parasite, and partly on the quantity and manner of distribution of the tannin mass.

Previously published data show that the susceptibility of Ribes species to blister-rust disease must be attributed to physiological rather than to anatomical characteristics.

Quantitative data for the seasonal tannin content of leaves, current-season stems, roots, and old stems of six far western Ribes species showed decreasing amounts of tannin for these plant parts in the order named. The six species included in these seasonal analyses were: R. petiolare, R. inerme, R. viscosissimum, R. lacustre, R. novadense, and R. roezli. In general, there was an increase in the tannin content of these Ribes species up to the mid-portion of the growing season. Additional data are given for single collections of leaves of R. petiolare, R. inerme, R. viscosissimum, and R. lacustre (Idaho): for leaves, stems and roots of R. bracteosum, R. erythrocarpum, and R. lacustre (Oreg.): and for leaves of R. nigrum and R. alpinum. Although the leaves of the highly susceptible black currants tended to be low in tannin, the present investigation showed that the quantity of tannin per se cannot be used alone to determine the susceptibility of ribes to blister-rust disease. The chemical nature of the tannins, particularly the type of protective substances which might be formed through interaction of enzymes and tannins, the location of the tannin-bearing cells, and the available food supplies near the point invaded by the parasite must all be considered.

A study of the decomposition products of the tannin mass of R. petiolare (highly susceptible) and R. inerme (moderately susceptible) showed the latter to contain more of the catechol tannins, which probably contribute to a higher specific toxicity to the blister-rust fungus, than those of R. petiolare, which were predominantly those of the gallotannin type. Differences were also noted in the ratio of alcohol to water-soluble tannins between R. petiolare on the one hand and a group of less susceptible ribes (R. inerme, R. lacustre, and R. viscosissimum) on the other.

Microchemical tests for tannin in leaves of Ribes petiolare, R. inerme, and R. lacustre showed that the tannins were concentrated in epidermal layers and around vascular bundles so as to facilitate a protective action.

Because of the clearly established wide variation in susceptibility of different Ribes species the ease with which a pure stock of ribes plants may be maintained under greenhouse conditions, and the practicability of making single spore inoculations, it is concluded that ribes and the blister-rust fungus have special advantages for research on the mechanism of disease resistance in plants.

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